Project title: Calcium Carbonate Production by Coccolithophorid Algae in Long Term, Carbon Dioxide Sequestration

Type of Report: Quarterly Progress Report #17
Reporting Period Start Date: April 1, 2006
Reporting Period End Date: June 30, 2006

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Date Report Was Issued: July 30, 2006

DOE Award Number: DE-FC26-01NT41132

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Abstract

Predictions of increasing levels of anthropogenic carbon dioxide (CO_2) and the specter of global warming have intensified research efforts to identify ways to sequester carbon. A number of novel avenues of research are being considered, including bioprocessing methods to promote and accelerate biosequestration of CO_2 from the environment through the growth of organisms such as coccolithophorids, which are capable of sequestering CO_2 relatively permanently.

Calcium and magnesium carbonates are currently the only proven, long-term storage reservoirs for carbon. Whereas organic carbon is readily oxidized and releases CO_2 through microbial decomposition on land and in the sea, carbonates can sequester carbon over geologic time scales. This proposal investigates the use of coccolithophorids — single-celled, marine algae that are the major global producers of calcium carbonate — to sequester CO_2 emissions from power plants. Cultivation of coccolithophorids for calcium carbonate ($CaCO_3$) precipitation is environmentally benign and results in a stable product with potential commercial value. Because this method of carbon sequestration does not impact natural ecosystem dynamics, it avoids controversial issues of public acceptability and legality associated with other options such as direct injection of CO_2 into the sea and ocean fertilization. Consequently, cultivation of coccolithophorids could be carried out immediately and the amount of carbon sequestered as $CaCO_3$ could be readily quantified. The significant advantages of this approach warrant its serious investigation. The major goals of the proposed research are to identify the growth conditions that will result in the maximum amount of CO_2 sequestration through coccolithophorid calcite production and to evaluate the costs/benefits of using coccolithophorid cultivation ponds to abate CO_2 emissions from power plants.

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Introduction

The objective of this project is to determine the efficacy of using coccolithophorid $CaCO_3$ production in CO_2 removal technology. This project will determine the methods and biological and chemical conditions needed to optimize the native ability of coccolithophorid algae to sequester CO_2 in the form of $CaCO_3$. This project will identify the parameters necessary to produce coccolithophorid blooms and the factors required to obtain maximum calcification rates. The information gained in this study can be incorporated into the design and construction of future algal ponds or bioreactors in follow-up research (not a part of this project) on CO_2 sequestration by coccolithophorids. This report describes progress made towards developing a method for separating calcareous coccoliths from cell organic matter such that the coccoliths may be recovered for biomedical and/or industrial uses.

Experimental

In completing our final milestone, we conducted experiments to develop a reliable method for detaching the calcareous coccoliths from the cell surface and separating these coccoliths from organic matter in the coccolithophorid *Emiliania huxleyi*. Our goal was to eliminate as much organic matter as possible, but to preserve the surface microstructure of the calcareous coccoliths which may have a variety of industrial uses.

We used a procedure modified from Paasche et al., 1996. First. we grew cultures in f/50 media (Guillard, 1975) under conditions described in previous reports. Subsequently, cell cultures were subsampled and centrifuged at 1000 rpm for 10 minutes. The resulting pellet was resuspended in 3 ml of supernatant. A 1% solution of Triton X-100 in 0.05 M $NaHCO_3$ was added to the resuspended pellet. Two drops of commercial NaOCl (ca. 8%) was added. The flask was then placed on rotating shaker at 100 excursions min^{-1} for 30 minutes. The experimental control consisted of a subsample centrifuged and resuspended in the same manner. However, in place of the addition of Triton X-100 and NaOCl, only growth media was added to the flask. Control flasks were placed on a rotating shaker at 100 excursions min^{-1} for 30 min, similar detergent-treated cell samples.

Results and Discussion

Examination by light microscopy revealed that the Triton X-100 treatment solubilized many, but not all, cells. More importantly, however, the detergent treatment caused the individual coccoliths to become detached from the cell surface. Few or no cells retained any calcareous coccoliths on their surface after this treatment. In contrast, samples which were centrifuged but not treated with detergent did not lose their coccolith coverings. Moreover, cell samples that were treated with Triton-X yielded solutions with coccolith

suspensions of evenly distributed coccoliths, with little or no clumping. In the cell sample that was not exposed to Triton X-100, clumps of cells with calcareous coccoliths dominated the sample. Thus, separation of the calcareous coccoliths from organic matter would not have been possible in the untreated sample. Our results suggest that the Triton-X solution combined with the NaOCl may have digested any organic coverings of the individual coccoliths which resulted in their detachment from the cell surface.

Conclusion

A modified method was used to detach coccoliths from the cell surface of coccolithophores and digest organic matter. Examination with light microscopy revealed the method to be highly effective. Because of the small size of *E. huxley*i coccoliths (5-7 μm), however, additional examination with the high resolution of scanning electron microscopy is necessary to confirm these results. Our future work will entail synthesizing results from this project and drafting a final project report.

References

Guillard, R.R.I. (1975) In: Smith, WH & Chanley, MH (eds.) *Culture of Marine Invertebrate Animals*. Plenum. New York, 726 pp.

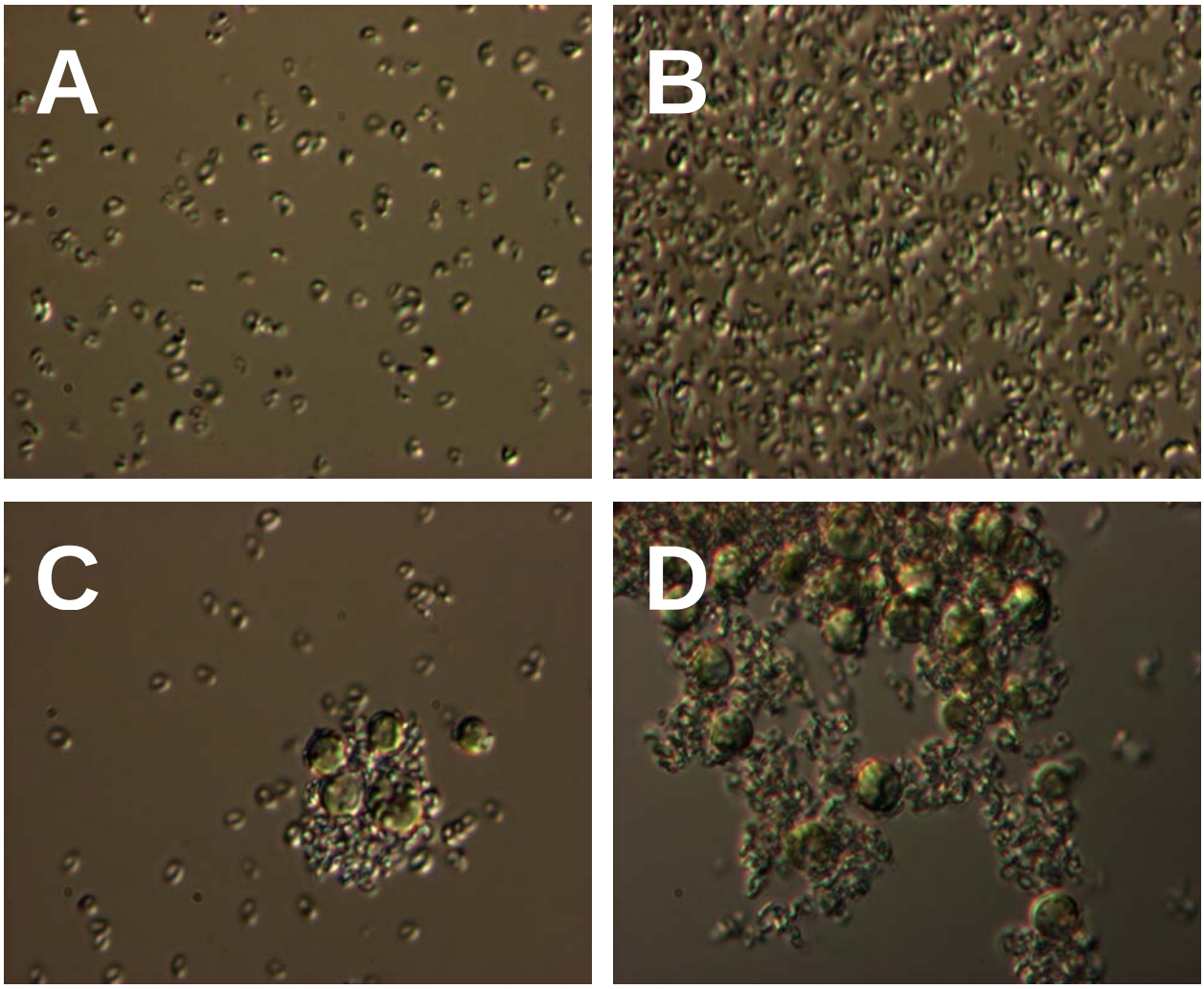

Figure 1. Light microscope photographs of the coccolithophore *Emiliania huxleyi* (magnification = 400X) illustrating the results of the detergent treatment to separate calcareous coccoliths from cells. **A and B**: Samples treated with Triton X detergent method to detach individual coccoliths and digest organic matter. Note individual coccoliths are not clumped. **C and D**: Control samples showing coccoliths still attached to cells and clumps of cells and coccoliths.